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Enterococci as a key parameter for water quality index: Purires River, Costa Rica

Luz Chacón, Víctor Arias, Kenia Barrantes, Wilson Beita-Sandí, Liliana Reyes and Rosario Achí

ABSTRACT

This study used the Canadian Water Quality Index (CWI) to characterize water sampled at three points within the Purires River micro basin, Costa Rica. The first sampling point is located in a high zone with domestic agricultural activities, the second point around the mid-point of the flow of the river, and the third point at the lowest zone with extensive agricultural activities mainly centered on the production of fresh vegetables. Eleven physicochemical parameters (As, Cd, Cr, BOD, COD, NH_4^+, NO_3^-, Pb, pH, PSO, and TSS) and two microbiological parameters (fecal coliforms and enterococci) were evaluated. We evaluated three different Canadian Water Quality Indexes (CWIs): CWI-1 included only physicochemical parameters, CWI-2 included CWI-1 parameters plus fecal coliforms, and CWI-3 included CWI-2 in addition to enterococci. Statistical analysis of individual parameters showed significant differences between sampling sites. CWI-1 was unable to discriminate between the three sampling points, and characterized the water quality as 'fair'. CWI-2 was only able to discriminate when the water contained high levels of chemical and microbiological contaminants, while CWI-3 adequately discriminated water quality at each of the sampling points. The evaluation of enterococci together with more traditional water quality parameters enabled better categorization of surface water quality.

Key words | Canadian water quality index, enterococci, fecal coliform, physicochemical parameters, surface water, water quality

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INTRODUCTION

Water quality has important implications for health, and poor water quality is linked to transmitted diseases such as diarrhea, cholera, dysentery, and parasitic and viral infections (Leclerc *et al.* 2002; Katukiza *et al.* 2013; Rosado-García *et al.* 2017). More than 842,000 diarrhea-associated deaths in low- and middle-income countries (LMICs) are related to inadequate water, sanitation, and hygiene conditions (Prüss-Ustün *et al.* 2014). In the United States (USA), the number of drinking-water gastrointestinal cases ranges from 12 to 19 million annually (Ashbolt 2015). Municipal surface water supply is implicated in 26 million infections and 13 million illnesses per year (Reynolds *et al.* 2008).

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Horton (1965) introduced the concept of an index to evaluate the status of water quality (WQI) in rivers by selecting the most commonly used water quality variables, such as dissolved oxygen (DO), pH, coliforms, specific conductance, carbon chloroform extract (CCE), alkalinity, and chloride content. A WQI allows the quantification of water quality by reducing a large number of physical, chemical, and biological variables to a single, dimensionless index in a reproducible form (Cude 2001). Subsequently, several WQI have been developed, some for general assessment of water quality (Sutadian *et al.* 2016) and others with specific applications such as recreational water, aquaculture, or water pollution (Neary *et al.* 2001; Abrahão *et al.* 2007;

Kannel *et al.* 2007; Sánchez *et al.* 2007; Almeida *et al.* 2008). The Canadian Water Quality Index (CWI), developed by the Canadian Council of Ministers of the Environment (CCME), is utilized not only in Canada but also in several other countries worldwide. It allows flexibility in selecting parameters, with a minimum of any four variables sampled a minimum of four times, and is therefore easily modified and adapted to regional conditions (Saffran *et al.* 2001).

Recently, Sutadian *et al.* (2016) compared 30 different WQI indexes, ten of which were based exclusively on physical and chemical parameters; 19 considered one microbiological parameter (e.g., fecal coliform, total coliform or *Escherichia coli*), and only one included four microbial parameters (fecal coliform, total coliform, *Escherichia coli*, and enterococci). Six of the seven most popular indexes, based on the number of applications in peer-reviewed journals and/or most widely used by government agencies, incorporated a bacteriological indicator; however, all of them used pre-assigned unequal weightings. Consequently, different weightings could lead to result biases, as the sensitivity of the final index to the most heavily weighted parameter, and a high concentration of microorganisms, may not be reflected in the final index. This is not desirable for a WQI that is intended to give a general status of water quality (Sutadian *et al.* 2016).

Generally, WQIs have three limitations: ambiguity, eclipsing, and rigidity (Swamee & Tyagi 2007). Ambiguity occurs when water quality sub-indexes show acceptable water quality for a specific use but give a final aggregated index that is out of range. Eclipsing occurs when the index fails to reflect the poor quality of one or more parameters. Finally, rigidity occurs when the index does not allow the inclusion of additional parameters, so that water may receive a good rating yet present a substandard water parameter that cannot be included in the assessment. The CCME-WQI does not utilize an index aggregation system, and its open system approach avoids rigidity; nevertheless, an eclipsing problem may occur when the index fails to reflect the poor quality of one or more of the included parameters.

Adequate classification of surface water is essential to determine the use of this resource. An interesting study case is the Purires River micro basin. Located in Cartago province, Costa Rica, at 83°55′ W and 9°54′ N, the surface area of the river is 76.25 km^2 and its length is 14.4 km. The maximum height is 2,200 meters above sea level (MASL) and the minimum is 1,380 MASL (Figure 1). The Purires river micro basin is located in the superior section of the Reventazón-Parismina hydrographic system (2,950 km^2) that leads to the Caribbean Ocean. The Reventazón-Parismina Basin is one of the most important hydrographic systems in Costa Rica, due to its generation of 25% of the hydroelectric power supply of the country, and the fact that it delivers water to nearly 2,500,000 inhabitants of the Greater Metropolitan Area of Costa Rica (Córdoba-Peraza 2015; Estado de la Nación 2015).

Land use in the study area is as follows: secondary forests (41.3%), pastures and deforested areas (25.2%), agriculture and urban development (16.4%), and greenhouses for vegetables and ornamental plant production (6.7%) (Córdoba-Peraza 2015).

The high-altitude zone of the Purires river is characterized by agriculture activities and patchy forest coverage. The main source of pollution is from sediments from soil runoff, fecal material, and gray waters from small urban settlements; however, the river shows pristine surface water characteristics. Barrantes *et al.* (2013) demonstrated the presence of fecal coliforms from 20 to 1,000 MPN/100 mL at this location.

The lower area is a flat landscape cultivation zone with extensive agricultural production of fresh vegetables, ornamental plants, and coffee and corn plantations, as well as cattle grazing activities. In addition, urban expansion and industrial developments are also important (Veas Atalya 2011). Water contamination arises from the agricultural, domestic, and industrial activities. As a consequence, the river water shows high turbidity and high fecal contamination with more than 5,000 MPN/100 mL, throughout the year (Barrantes *et al.* 2013).

Fecal coliforms, *Escherichia coli* and enterococci, are usually considered appropriate indicators of fecal water contamination of human or animal origin. A study by Wu *et al.* (2011) of surface water shows that no single microbial indicator was identified as the most correlated with pathogen presence in water, however, enterococci were more likely to be correlated with pathogens, above other indicators. Using surface water samples from urban catchments in Singapore, Liang *et al.* (2015) found a significant positive correlation between *Salmonella* and *Pseudomonas aeruginosa*

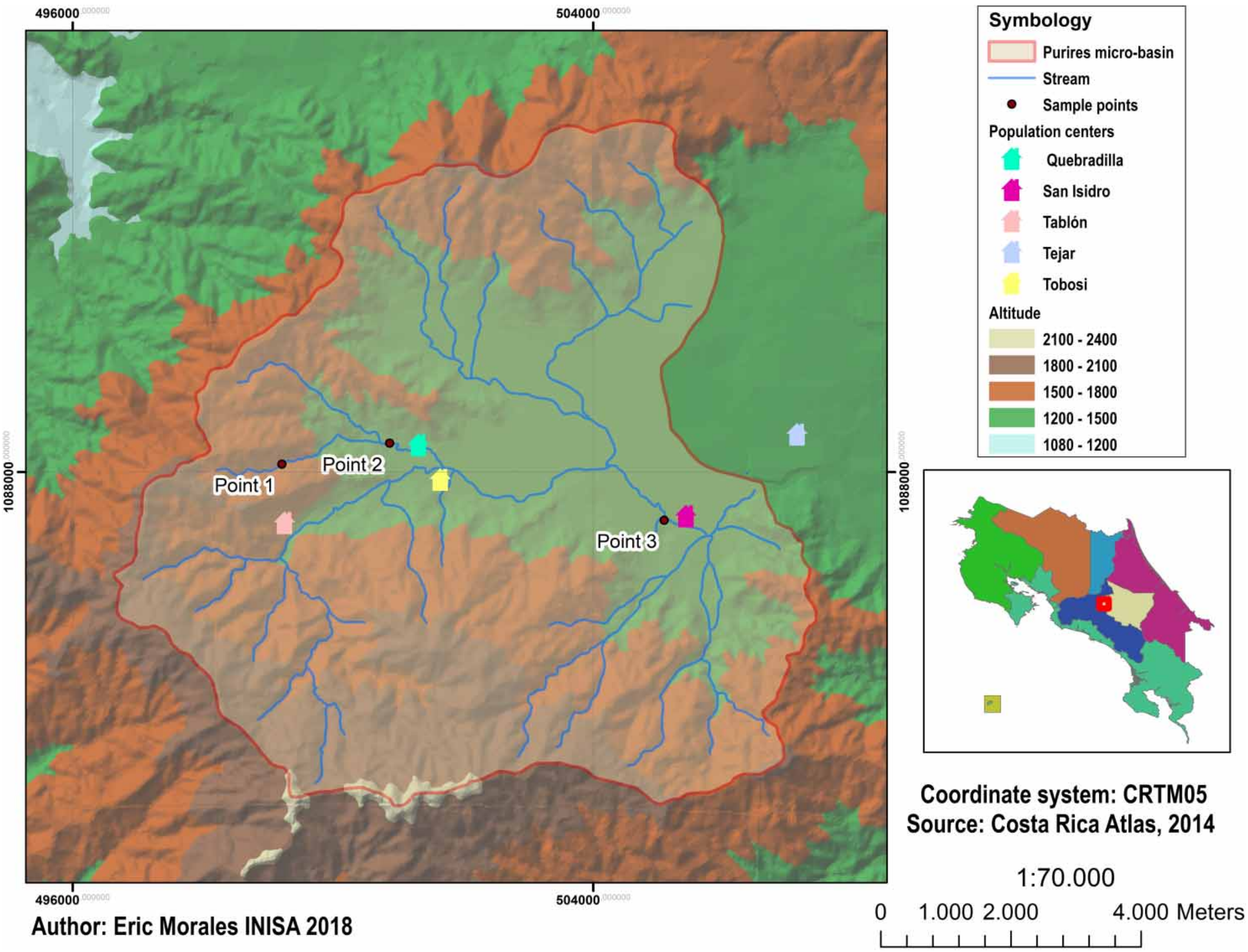

Figure 1 | Geographical information of Purires River micro basin.

with most of the microbial indicators, especially *E. coli* and enterococci.

In the present study, water samples from the Purires River were used to determine the suitability of a Water Quality Index as a tool for evaluating water quality according to intended agricultural use. The principal aims of this study were to demonstrate a simple procedure to overcome the eclipsing problem that may be present in the CCME-WQI and to evaluate the usefulness and relevance of incorporating enterococci as an indicator of fecal contamination in the CWI. In this report, we also aim to highlight the fact that health risks might be overlooked if only chemical parameters are taken into account or if considering only *E. coli* in microbiological risk assessment.

METHODS

Sampling

The Purires River micro basin is located in the province of Cartago, Costa Rica, and has an area of 76.25 km^2. Water samples were collected monthly at three sampling sites from September 2011 to August 2012, the period was influenced by the La Niña phenomena (Comisión Técnica Cunsultiva Nacional del Fenómeno ENOS 2010). Sampling points are located as shown in Figure 1. Sampling site 1 is located at the high zone, 1,599 MASL, 84°01′ W and 9°83′ N; Sampling site 2 is located in a middle zone, is at 1,415 MASL, 83°99′ W and

9°85′ N; and sampling site 3 is the lowest, at 1,381 MASL, 83°95′ W and 9°83′ N (Figure S1, see Supplementary Information). Temperature, pH, and dissolved O_2 were measured on site. Water samples were collected at each site in 200 mL previously sterilized glass bottles (20 min at 121°C and 100 kPa) for microbiological analysis, transported at 4°C to the laboratory, and analyzed within 8 hours of collection. Additional water samples were collected in 2 L plastic bottles for physical and chemical analysis.

Microbiological and physicochemical analysis

The following parameters were analyzed using the indicated Standard Methods (APHA 2005): Fecal coliforms and enterococci were determined by most probable number (MPN/100 mL) (9221E and 9221 F, respectively), pH (4500-H^+ B), percent saturation of dissolved oxygen (PSO) (4500-O G), total suspended solids (TSS) (2540 C), biochemical oxygen demand (BOD) (5210 B), chemical oxygen demand (COD) (5220 D), nitrates (NO_3^-) (4110 B), ammonium (4500-NH_3 F), and arsenic, cadmium, chromium, and lead (3113 B). The limits of quantification (LOQs) for the water characterization parameters are given in Table S2 (see Supplementary Information).

Water quality indexes

The CWI was calculated according to Neary *et al.* (2001) using three approaches. The maximum acceptable ranges used for calculating the different indexes are summarized in Table 1. In the first approach, the index (CWI-1) was calculated by using only physicochemical variables, specifically ammonium, arsenic, cadmium, chromium, BOD, COD, lead, nitrates, PSO, pH, and TSS. A second index (CWI-2) consisted of the above-mentioned physicochemical variables plus fecal coliforms. Thirdly, index CWI-3 included the physicochemical variables of CWI-1 and fecal coliforms from CWI-2 in combination with fecal enterococci (Table 2). The calculated CWIs were categorized as recommended by the CCME: Excellent (95–100): water quality protected with absence of threat or impairment; Good (80–94): water quality with only a minor degree of threat or impairment; Fair (65–79): water usually protected but occasionally threatened or impaired; Marginal (45–64): water frequently threatened or impaired; and Poor (0–44): water quality almost always threatened or impaired (Neary *et al.* 2001).

Table 1 | Accepted ranges for physicochemical and microbiological parameters for surface water, and their reference methods

Parameter	Limit range	Source
Ammonium (mg/L)	<0.5	Calvo & Mora (2007)
Arsenic (mg/L)	<0.05	EPA (2015)
Cadmium (mg/L)	≤0.009	EPA (2015)
Chromium (mg/L)	≤0.05	EPA (2015)
COD (mg/L)	<25	Calvo & Mora (2007)
BOD (mg/L)	≤3	Calvo & Mora (2007)
Fecal coliforms (MPN/100 mL)	<800	EPA (2015)
Fecal enterococci (MPN/100 mL)	<800	Same as fecal coliforms EPA (2015)
Lead (mg/L)	≤0.09	EPA (2015)
Nitrate (mg/L)	<10	EPA (2015)
pH 20.0 °C	6.5–8.5	EPA (2015)
POS (%)	91–100	Calvo & Mora (2007)
Total suspended solids (mg/L)	<1,000	EPA (2015)

Statistical analysis

In order to determine the importance of animal fecal pollution, the ratio of fecal coliform to fecal enterococci was calculated according to the method described by the World Health Organization; a ratio of four or greater may indicate human pollution, ratios of two or less may indicate animal pollution (Ashbolt *et al.* 2001). Significant differences between each parameter and between sampling sites were tested using a non-parametric Kruskal–Wallis test and adjusted Nemenyi test for post-hoc analysis, with $p = 0.05$ and 95% of coverage. A Pearson's correlation matrix was calculated to evaluate the correlations between different physicochemical and microbiological parameters, with 0.60 as the minimum significance value and using the chi-square test for statistical significance ($p = 0.05$). All statistical tests were conducted in *R* software (version 3.2.0, 2015-04-16).

Table 2 | Physicochemical and microbiological parameters according to sampling site at the Purires River, Costa Rica

	Sampling sites		
	Site 1	Site 2	Site 3
Ammonium[a]	0.06^{1}	0.06^{1}	$(2.3 \pm 2.2) \times 10^{1}$
Arsenic[a]	$(4.0 \pm 2.0) \times 10^{-3}$	$(4.0 \pm 0.3) \times 10^{-3}$	$(5.0 \pm 0.6) \times 10^{-3}$
Cadmium[a]	0.0001^{1}	0.0001^{1}	0.0001^{1}
Chromium[a]	0.001^{1}	0.001^{1}	0.001^{1}
BOD[a]	4.1 ± 3.7	6.7 ± 7.3	$(1.1 \pm 1.5) \times 10^{1}$
COD[a]	30.0^{1}	30.0^{1}	$(4.5 \pm 4.1) \times 10^{1}$
Enterococci[b]	$(7.8 \pm 6.5) \times 10^{2}$	$(1.9 \pm 4.5) \times 10^{4}$	$(2.3 \pm 1.9) \times 10^{4}$
Fecal coliforms[b]	$(9.1 \pm 5.5) \times 10^{2}$	$(9.4 \pm 9.4) \times 10^{3}$	$(6.4 \pm 9.5) \times 10^{4}$
Nitrate[a]	$(6.5 \pm 3.7) \times 10^{-1}$	$(9.0 \pm 6.9) \times 10^{-1}$	$(2.9 \pm 0.9) \times 10^{1}$
POS[a]	$(8.3 \pm 0.4) \times 10^{1}$	$(8.2 \pm 0.4) \times 10^{1}$	$(6.1 \pm 1.2) \times 10^{1}$
pH	7.9 ± 0.2	7.8 ± 0.2	7.6 ± 0.2
TSS[a]	$(1.4 \pm 0.9) \times 10^{1}$	$(3.0 \pm 4.3) \times 10^{1}$	$(1.0 \pm 1.6) \times 10^{2}$

[1]$p = 0.05$. Units: [a]mg/L; [b]MPN/100 mL.

RESULTS

Mean monthly values for water quality parameters at the three study sites are shown in Table 2. A Kruskal–Wallis test was conducted to determine whether there were differences in parameter concentrations between the three sampling sites (Table 3). Median concentration was significantly different between the sites for ammonium, nitrate, oxygen saturation, pH, fecal coliforms, and enterococci. Subsequently, pairwise comparison was performed using the *p*-adjusted Nemenyi test for multiple comparisons. Adjusted *p*-values are presented. This post-hoc analysis revealed significant differences in median concentration between sites 1 and 3, and 2 and 3 for ammonium, nitrate, oxygen saturation, and pH. For fecal coliforms, there were significant differences between sites 1 and 2 and 1 and 3. For enterococci, a statistically significant difference was found only between sites 1 and 3 (Table 3). In most cases, the lowest concentrations occurred at site 1 and the highest concentrations at site 3. Furthermore, the concentrations of arsenic, cadmium, chromium, and lead were below the LOQs in most of the samplings throughout the year. The LOQs were used for the computation of the CWI.

Table 3 | Statistical significance of analyzed parameters according to sampling site

Parameter analyzed	*p* Kruskal–Wallis[1]	*p*-adjusted Nemenyi test[1,a]		
		1–2	1–3	2–3
Ammonium	$\mathbf{3.949 \times 10^{-7}}$	1.000	$\mathbf{3.700 \times 10^{-04}}$	**0.004**
Arsenic	0.866	0.940	1.000	0.950
BOD	**0.030**	0.660	0.210	**0.030**
COD	0.128	1.000	0.760	0.760
Enterococci	**0.007**	**0.084**	**0.007**	0.634
Fecal coliforms	$\mathbf{1.780 \times 10^{-6}}$	**0.006**	$\mathbf{1.000 \times 10^{-6}}$	0.113
Nitrates	$\mathbf{2.025 \times 10^{-5}}$	0.782	$\mathbf{5.800 \times 10^{-5}}$	$\mathbf{9.200 \times 10^{-4}}$
pH	**0.002**	0.977	**0.004**	**0.009**
POS	$\mathbf{1.536 \times 10^{-4}}$	0.560	**0.002**	**0.008**
TSS	0.164	1.000	0.220	0.250

[1]$p = 0.05$.
[a]Numbers 1, 2, and 3 refer to sampling sites for pairwise comparisons.

The Pearson correlation coefficient matrix for the various water parameters is shown in Table 4. Positive linear correlations were found between ammonium and fecal coliforms ($r = 0.74$), and between BOD and TSS ($r = 0.74$). Negative linear correlations were found between nitrate and both oxygen saturation ($r = -0.73$) and pH ($r = -0.62$), and between pH and TSS ($r = -0.69$). All variables with correlation coefficients $r > 0.6$ had *p*-values < 0.01.

Table 4 | Pearson correlation matrix for the studied data ($n = 36$, $^{**}p < 0.01$)

	Ammonium	Arsenic	BOD	COD	Nitrate	POS	pH	TSS	Fecal coliforms	Enterococci
Ammonium	1.00	0.28	– 0.07	0.07	0.58	– 0.56	– 0.24	– 0.05	****0.74**	0.30
Arsenic		1.00	– 0.18	– 0.10	0.16	– 0.30	0.14	– 0.12	0.06	0.11
BOD			1.00	0.21	0.29	0.06	– 0.47	****0.74**	– 0.08	– 0.11
COD				1.00	0.36	– 0.11	– 0.24	0.21	0.06	– 0.02
Nitrate					1.00	** – **0.73**	** – **0.62**	0.38	0.36	0.20
POS						1.00	0.44	– 0.05	– 0.44	– 0.24
pH							1.00	** – **0.69**	– 0.23	– 0.02
TSS								1.00	0.03	– 0.06
Fecal coliforms									1.00	0.32
Enterococci										1.00

Enterococcus analysis

Fecal enterococci were not related to any of the studied parameters ($p > 0.05$) (Table 4), so it was considered as a possible additional parameter for the analysis of water quality. Additionally, we calculated the fecal/enterococci ratio (useful for source tracking analysis) for each of the three sampling sites (Figure 2). The majority of the ratios (23 out of 36) were less than 2, indicating that contamination was likely of animal origin. Seven out of 36 presented a ratio greater than 4, indicative of probable human pollution. The remaining six samples were categorized in the undefined zone, with values between 2 and 5 (Ashbolt *et al.* 2001).

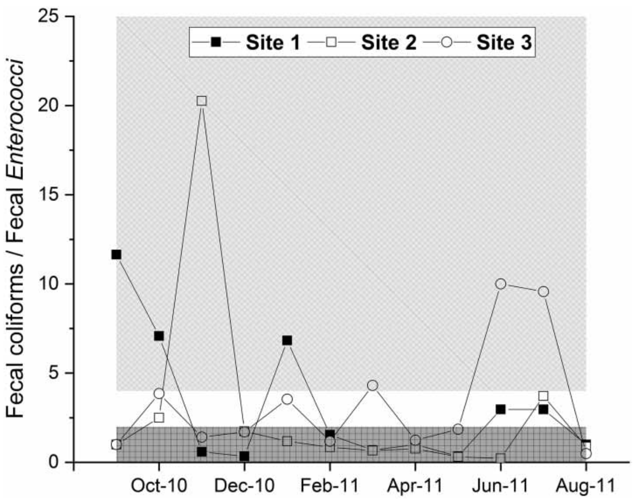

Figure 2 | Fecal coliform to fecal enterococci ratios by study site. Shading indicates pollution by possible human (light grey), animal (dark grey) and indeterminate (white) sources.

Canadian water quality indexes

CWI-1 and CWI-2

CWQ-1 was calculated using 11 physicochemical parameters. Water oxygenation was evaluated by PSO; BOD and COD were used to evaluate oxygen demand. Eutrophication was evaluated by the presence of nitrates and ammonium and the physical aspect by TSS and pH. CWI-2 included all the above-mentioned chemical parameters plus fecal coliforms, to measure the risk of water-transmitted microbial infections.

As shown in Table 2, water quality from the three sampling sites was categorized as 'fair' according to CWQ-1. The inclusion of fecal coliforms in CWI-2 resulted in site 3 being assessed as 'poor', and the overall river quality as 'marginal'; Site 2, with a high concentration of fecal coliforms (mean 9,400 MPN/100 mL), remained categorized as 'fair'.

CWI-3

This index was obtained by adding enterococci to the CWI-2 parameters. The only accepted standard range for enterococci is for recreational water use, 130 CFU/mL (EPA 2012). However, a recent meta-analysis of the literature by

McMinn et al. (2017) found similar concentrations of fecal coliforms ($\log_{10}$ 3.46 ± 1.41 CFU per 100 mL) and enterococci ($\log_{10}$ 3.00 ± 1.47 CFU per 100 mL) in freshwater, with a 95% confidence limit. Therefore, in the present case, the accepted concentration for fecal coliforms, which is <800 MPN/100 mL in surface water (EPA 2015), was used as a parameter for enterococci as well. Of the 36 water samples, eight samples from site 1 (highest sampling point) and one from site 2 (intermediate sampling point) had enterococci values within the acceptable range (Table 1). Site 1 had the lowest mean values for fecal coliforms (910 MPN/100 mL) and enterococci (779 MPN/100 mL). Using CWI-3, site 1 with low fecal coliforms and low enterococci was evaluated as having 'fair' water quality. Sites 2 and 3, both with high fecal coliform concentrations and enterococci out of range, were categorized as 'marginal' and 'poor', respectively (Figure 1 and Table 5).

DISCUSSION

Water quality is influenced by climatological and geological factors such as temperature, forest coverage, rainfall, and runoff elements from soil, and it is also impacted by human activities, particularly the discharge of organic and inorganic wastewater products, agricultural runoffs (e.g., fertilizers), and sewage discharges. Concentrations of ammonium, BOD, nitrate, and TSS in water are used as indicators of organic matter pollution and the impacts of sewage release into rivers (WHO 2006; Bilotta & Brazier 2008). BOD indicates high concentration of organic pollution and high concentration of biodegradable organic material in water. Ammonium occurs at high concentration in sewage; when present in stream water, ammonium utilizes the available oxygen for oxidation process to nitrate. TSS has high organic content and its decomposition can deplete levels of dissolved oxygen, resulting in critical oxygen shortage. Chemical analysis of the Purires River samples indicated that water pollution increased downstream, as shown by the statistically significant differences in ammonium, BOD, nitrate, and TSS from sites 1 through 3 (Tables 3 and 4). The river water is mainly used for farming activities, irrigating vegetables, livestock husbandry, and greywater discharges (Córdoba-Peraza 2015). The pollutants observed at site 1 were attributed to the presence of scattered agricultural and dairy farms; and at sites 2 and 3, the high chemical and microbial contamination were attributed to runoff water, the increasing number of farms, and to urban and industrial activities, and the convergence of other tributary streams.

Microbiological contamination also increased downstream, with the lowest concentrations of fecal coliforms and enterococci found at site 1 and high concentrations of microorganisms at sites 2 and 3 (Table 3), which was in agreement with the chemical water indicators of organic matter pollution.

Under Costa Rican legislation, surface water used in food production must comply with a range of chemical, organic, and microbiological parameters (Class 2 water). According to this standard, surface water with fecal coliform levels >1,000 MPN/100 mL cannot be utilized in food production unless treated by conventional methods (MINAE 2007). As shown in Table 2, only site 1 had a majority of samples (8 out of 12) that met this standard; levels were significantly higher at sites 2 and 3, and always exceeded this range (Table 3); however, surface waters from site 2 and 3

Table 5 | Canadian Water Quality Indexes for sampling sites on the Purires River

	CWI-1		CWI-2		CWI-3	
	Index	**Descriptor**	**Index**	**Descriptor**	**Index**	**Descriptor**
Site 1	79.71	Fair	75.92	Fair	73.09	Fair
Site 2	78.32	Fair	61.81	Fair	48.81	Marginal
Site 3	62.56	Fair	39.05	Poor	36.10	Poor
Global[a]	71.59	Fair	56.97	Marginal	43.54	Poor

[a]Global: this index includes data from all sampling points on the Purires river and reflects a general status of water quality. Descriptor ranges: Fair (65–79); Marginal (45–64); Poor (0–44).

are regularly used for illegal irrigation of fresh vegetables (Astorga 2007).

As recommended in the CWI (Neary *et al.* 2001), the application of an index to water quality should utilize a set of parameters relevant to the water tested and its intended use. To accomplish this, three water quality indexes were formulated to provide good discrimination between sampling sites and in accordance with the intended use of irrigation.

When applying CWI-1, water quality was categorized as 'fair' at all three samples sites, which implies that water quality sometimes deviated from natural or desirable levels (Neary *et al.* 2001). Nevertheless, this index did not discriminate between low-agricultural areas (site 1) and those that display the effects of agricultural practices and livestock rearing (sites 2 and 3). Although this chemical index is the most widely used worldwide (Sutadian *et al.* 2016), it omits the health risks associated with microbial presence (Cabral 2010; Baldursson & Karanis 2011; Prüss-Ustün *et al.* 2014; Dickin *et al.* 2016). CWI-2 gave better discrimination between site 1 (rated as 'fair') with low-intensity agriculture, and site 3 (rated 'poor'), impacted by high-intensity agriculture and urbanization. However, CWI-2 failed to reflect the poor microbiological water quality of site 2 (rated 'fair'), thereby eclipsing the significantly high fecal coliform count at site 2 in comparison with sites 1 and 3 (Table 3). By including enterococci, CWI-3 overcame this eclipsing limitation and allowed discrimination of sites 1 ('fair'), 2 ('marginal'), and 3 ('poor').

The Produce Safety Rule of the Food Safety Modernization Act (FDA 2017), prohibits the use of untreated surface water for any procedure in which there is a reasonable chance for the transfer of pathogenic microorganisms to products through direct or indirect contact. In addition, to safeguard products from microbial contamination with unreliable surface water supplies, irrigation water quality guidelines and regulations have been issued by countries such as Canada that limit bacterial presence to $<1,000$ fecal coliforms per 100 mL, similar to the Costa Rican legislation (MINAE 2007; Uyttendaele *et al.* 2015).

Moreover, the WHO stated that wastewater with fecal coliform concentrations of more than 10^4 thermo-tolerant coliforms per 100 mL was associated with increased non-specific diarrhea infection (Blumenthal *et al.* 2000; WHO 2006). Surface water from the Purires River is not categorized as wastewater; however, the illegal agricultural use of the surface water with high concentrations of fecal coliforms found at sites 2 and 3 carries associated health risks that must be reflected in a water quality index.

Fecal-contaminated water poses a health risk in the production of leafy vegetables and vegetables eaten raw (Allende & Monaghan 2015). Furthermore, all around the world, the consumption of fecal-contaminated fresh produce is linked to foodborne disease outbreaks, a portion of which probably originate from poor-quality water use in production, processing/packing, and preparation (Alegbeleye *et al.* 2018). In addition, irrigation with contaminated water is a mechanism for transmission of microbial, viral, and parasitic diseases (Mazari-Hiriart *et al.* 2008; Allende *et al.* 2017). In Costa Rica, Calvo *et al.* (2004) reported the presence of *Cyclospora* sp., *Cryptosporidium* sp., microsporidia, and fecal coliform in vegetables and fruits, acquired in local agricultural markets that had been irrigated with untreated surface water.

The next step to ensure both safety of fresh produce for consumers and compliance with water quality standards, is the implementation of preventive control measures to avoid or mitigate contamination of irrigation water (Allende & Monaghan 2015; Markland *et al.* 2017). Application of water monitoring plans for fecal indicator bacteria; physical disinfection of irrigation water by ultrasound, ultraviolet, or filtration treatments; or chemical disinfection with chlorine-based sanitizers, are the most commonly used (Allende & Monaghan 2015; Uyttendaele *et al.* 2015). Alternative production systems (furrow irrigation, hydroponic culture, and nutrient film techniques), and when feasible, transfer of the water catchment source to a non-contaminated site, are other possibilities. Some of these strategies can be implemented at the Purires River site 3, for example, monitoring of fecal indicator bacteria and chemical disinfection of irrigation water, with promising results.

CONCLUSIONS

The proposed CWI-3 index with the inclusion of As, Cd, Cr, BOD, COD, fecal coliforms, NH_4^+, NO_3^-, Pb, pH, PSO, TSS and especially enterococci, achieved the goal of allowing appropriate discrimination of irrigation water quality and evaluation of the health risks associated with water from each sampling site. The evaluation of enterococci levels in combination with traditional water quality parameters enables improved categorization of surface water quality, successfully adapting the flexible Canadian Water Quality Index (CWI) to suit the context of the study area. The CWQ-3 is an example of the importance and necessity of the inclusion of more than one microbial parameter in a water quality index analysis; the conventional index calculation (with only one microbial parameter) can eclipse the real health risk associated with the use of water from a contaminated site in a prohibited activity such as the irrigation of fresh vegetables.

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